1818-Pos
Mechanical Properties of Human Cardiac Tropomyosin in Familial Hypertrophic Cardiomyopathy (FHC) Probed by Atomic Force Microscopy
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α-Tropomyosin (αTm) is a 66kDa alpha-helical coiled-coil protein in thin filaments of cardiac muscle. Together with the Troponin complex (Tn), it is responsible for Ca^{2+} regulation of striated muscle contraction. When Ca^{2+} is released from sarcoplasmic reticulum and bound to Tn, the complex undergoes a conformational change that moves Tm away from myosin binding sites on actin, sites that are blocked in the absence of Ca^{2+}. This allows actomyosin cross-bridge cycling, resulting in force generation and/or sarcomere shortening. A Tm mutation (E180G) found in some FHC patients results in altered thin filament function including enhanced Ca^{2+} sensitivity. This study seeks to investigate at the single molecule level our hypothesis that the E180G mutation alters mechanical properties of αTm. Recombinant, bacterially-expressed wild type (WT) and E180G mutant αTm's were deposited and dried on separate mica surfaces coated with poly-lysine. Topographical AFM images of the samples were taken in AC-mode (Asylum Research, Santa Barbara, CA) at a resolution of 0.5x0.5 nm^2/pixel. Images were analyzed in MATLAB to obtain estimates of single Tm end-to-end distances, which were normalized with the ratio between the measured and expected contour lengths. The normalized mean squared end-to-end distance ($<R^2>$) for the E180G mutant (N=309) was ~4% smaller ($P<10^{-3}$) than that for WT (N=307). $<R^2>$ of a linear molecule is related to its average flexural rigidity. Differences between WT and mutant may point to a mutation-induced alteration in local structure or flexibility of the molecule as a whole. This prompts for further study to investigate the nature of the observed change. Furthermore, a quantitative model relating end-to-end length distribution to the persistence length of αTm will be instrumental in understanding the molecular mechanics underlying FHC. Support: NIH and American Heart Association.

1819-Pos
EPR as a Tool to Study the Dynamic of Tropomyosin in the Muscle Fiber - Use of a Bifunctional Spin Label
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Tropomyosin (Tm), an alpha-helical coiled-coil protein, is a key regulatory protein in muscle contraction. Little is known about the role of Tm dynamics in muscle regulation and more specifically dynamics in the three states of the thin filament. In this work, the flexibility of four different regions of Tm was determined with Saturation Transfer Electron Paramagnetic Resonance (ST-EPR). The use of bi-functional labels allowed us to immobilize the probe and prevent the motion of the label with respect to protein surface. The rotational correlation time of the bi-functional spin label on Tm was 40 ns compared to 25 ns for a conventional mono-dentate spin label. The spin label was attached to i, i+4 positions of the coiled-coil, obtained by cysteine mutagenesis. The bi-functionally labeled Tm di-mutants were reconstituted into ''ghost muscle fibers'' from which the myosin filaments and intrinsic regulatory proteins (tropomyosin, troponin) were removed. The filaments were reconstituted with Tn and decorated with myosin S1. We found that there is a gradient of flexibility of Tm along its length in the muscle fiber with the C-terminus mutant A268C/E272C being less mobile (two-fold), as compared to the rest of the mutants. Introduction of troponin decreases the flexibility of the four mutants, specifically the two mid-region mutants H153C/D157C and G188C/E192C by 25% and 30% respectively. The addition of calcium did show a decrease in the flexibility of the C terminus mutant A268C/E272C by 20%. The effect of calcium addition on the two other mutants and the effect of addition of S1 on the four mutants were not significant. Thus, although there is a gradient of flexibility in Tm, its dynamics does not change within different states of thin filament activation.

1820-Pos
Comparison of the Conformational Stabilities of Striated Alpha-Tropomyosins Adapted to Different Temperature Regimes
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The conformational stabilities of alpha-striated tropomyosins from warm and cold blooded sources have been compared using circular dichroism (CD) and differential scanning calorimetry. Alpha-tropomyosins from rabbit and shark share 95% sequence identity. There are three replacements in the core: residues Thr179Ala, Ser190Cys and Ser211Ala. At low temperature (pH 7) the two tropomyosins are equivalent in molar ellipticity at 222nm, however, shark tropomyosin unfolds over a wider temperature range than the mammalian counterpart. The dependence of this ellipticity on temperature (0.1M salt, pH 7, 2mg/mL protein + dithiothreitol in the case of rabbit tropomyosin) is characterized by the following transitions: shark, ~33 (main, ~two-thirds of total change in signal) and ~54°C and rabbit, ~41 (main) and ~49°C. At ~10mg/mL, the ΔTm for shark tropomyosin becomes larger on account of an upward shift in the minor transition, otherwise the results of calorimetry are in agreement with those of CD. Analysis of fragments CN1A (residues 11-128, Tm ~59°C) and CN1B (residues 142 - 281, Tms ~20 and 35°C) by far-UV CD and intact protein by near-UV CD (Tm ~ 32°C), shows that the most stable section of shark tropomyosin is located in the amino-terminal half of the molecule, possibly the first ~100 amino acids. The enhanced flexibility (lower stability) of the remainder of the molecule coincides with the presence of a row of destabilising 'core' amino acids between residues 179 - 221. A final point is that conformational stability is insensitive to the presence of a covalently bound phosphate group, consistent with the structural disordering of the corresponding region of each tropomyosin.

1821-Pos
Tropomyosin and Troponin-T Isoform Differences in Jaw-Closing Muscles of Rodentia and Carnivora that Express Masticatory Myosin
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We recently reported that, contrary to prevailing dogma, masticatory (''superfast'') myosin is expressed in jaw-closing muscles of some members of Rodentia (Reiser et al., J. Exp. Biol. 212:2511-2519, 2009). Whereas virtually all mammalian limb muscle fibers express tropomyosin-β (Tm-β), along with fast-type or slow-type Tm-α, jaw-closing muscle fibers that express masticatory myosin in members of Carnivora express a unique isoform of Tm-α and do not express Tm-β (Rowlerson et al., Biochem. Biophys. Res. Commun. 113:519-525, 1983; Hoh et al., Proc. Aust. Physiol. Pharmacol. Soc. 20:192P, 1989). The goal of this study was to examine thin filament protein isoform composition in jaw-closing muscles of rodents that express masticatory myosin and compare the results to those from members of Carnivora. Tm or troponin-T (TnT) specific antibodies and immunoblotting were used to probe homogenates of limb and jaw-closing muscles of six species of Rodentia and three species of Carnivora. The results verify the almost exclusive expression of Tm-α, presumably the unique isoform reported earlier, in the jaw-closing muscles of Carnivora and reveal that members of Rodentia express both Tm-α and Tm-β in jaw-closing muscles, similar to limb muscles in the same species. The results also indicate that the same complement of TnT isoforms are expressed in jaw-closing and limb muscles of Carnivora, but differ markedly between the two muscle groups in Rodentia. Fast-type TnC and TnI appear to be expressed in jaw-closing muscles of both orders. It is postulated that the differences in Tm and TnT isoform expression patterns between Carnivora and Rodentia may impart fundamental differences in calcium-sensitivity of force generation to accommodate markedly different feeding styles, with shared expression of masticatory myosin, between these two animal orders. Supported by the National Science Foundation.

1822-Pos
What Region of Tropomyosin Interacts with the N-terminal Half of Troponin T?
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The thin filament of striated muscle is made up of troponin C, troponin I and troponin T (TnT) bound to actin- tropomyosin (actinTm). Previous photochemical cross-linking studies revealed that TnT interacts more closely with Tm near residue 174 than Tm near residue 146 (Mudalige et.al., JMB 2008).
To determine the cross-linking site of TnT with Tm174, photolysed thin filaments were subjected to limited chymotryptic digestion. SDS-PAGE showed the presence of a new band corresponding to a molecular weight close to the molecular weight of cross-linked Tm-TnT2 and Tm-TnT1 (where TnT2 and TnT1 are the two major chymotryptic peptides of TnT) suggesting that Tm174 cross-linked with either the TnT1 or TnT2 regions of TnT or both. From preliminary MALDI-TOF analysis of trypsin in-gel digestion of the Tm174-TnT band(s), two new tryptic peptides were observed corresponding to a Tm174 tryptic peptide linked to three possible TnT tryptic peptides: 1) 171-174; 2) 139-144; 3) 113-115. Further analysis will determine if Tm 174 in the C-terminal half of TnT is close to the TnT1 (1-159) or TnT2 (160-282) regions of TnT. However, a preliminary photolysis study with the cross-linker at Tm100 showed no cross-linked bands in contrast to Tm174, suggesting that the N-terminal half of Tm does not interact with TnT. Further studies are underway to confirm this proposal. Supported by NIH HL22461-29.